Faculdade de Medicina de Lisboa

NENS STIPEND FOR TRAINING STAYS

Charíté Universitätsmedizin Berlin

Report

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NENS STIPENDS FOR TRAINING STAYS

The training stay took place at the Institute of Neurophysiology in Charité - Universitätsmedizin in Berlin (Germany), from April 14th to May 18th. The primary goal of this training stay was to get familiarized with each step of the low-magnesium induction of seizure-like events procedure, in order to be able to establish it successfully in our laboratory (Institute of Pharmacology and Neurosciences, Faculty of Medicine, University of Lisbon, Portugal).

Preparation of hippocampal slices: Male and Female Wistar rats were decapitated under deep isoflurane anesthesia. The skull was exposed and cut along the sagital suture from the foramen magnum to the forehead with a pair of surgical scissors. On each temporal side of the skull, one cut was made at the foramen magnum. The brain was exposed by carefully prying the skull open. Holding the skull upside down and using a spatula to sever the cranial nerves that hold the brain to the skull, the brain was removed and submerged in ice-cold (4°C) aCSF, containing in (mM): 124 NaCl, 3 KCl, 1.8 $MgSO_2$, 1.6 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose. The solution was equilibrated with carbogen gas (95% O_2/5% CO_2) to maintain a pH of 7.4 and the osmolarity of the solution was about 290-300 mOsm. This procedure was performed within less than a minute as recommended. After isolation, the brain was kept at 0–4°C, since low temperatures reduce metabolism-related ischemic damage and improve texture for slicing. The cerebellum was removed, and the brain was divided into two hemispheres. Each hemisphere was placed on its medial surface and divided into rostral and caudal sections by a transverse cut in a plane approximately parallel to the main axis of the hippocampus.

A thin layer of cyanoacrylate glue was applied on the slicing stage of the vibratome (VT1200 S, Leica, Germany). Then the tissue was gently placed, with help of a w-shaped piece of filter paper, with dorsal plane fixed onto the glue. Aerated ice-cold aCSF was carefully poured in to the vibratome. The vibratome section thickness was adjusted to 400 µm, the frequency of vibration to the maximum and slow speed. Stainless steel razor blades (Gillette Platinum, UK) were cleaned with 75% ethanol and rinsed thoroughly with distilled water before use. Slices were immediately transferred onto lens cleaning paper (Kodak, USA) in a custom made interface chamber perfused with aCSF at 36 ± 0.5°C (flow rate: ~1.8 ml/min). Eight to ten horizontal slices were cut, containing the temporal cortex area, the perirhinal cortex, the entorhinal cortex, the subiculum, the dentate gyrus, and the ventral hippocampus were obtained. Slices were allowed to recover for at least 90 min, before starting the recordings.

Extracellular recordings and Low-Magnesium induced seizure-like events: Recording electrodes were made from borosilicate glass capillaries (GB 150F-8P*;* Science Products) in two stages on a

pipette puller (P-87 Flaming/Brown Puller, Sutter Instruments Co., USA), and filled with aCSF. This were placed simultaneously in area CA3 and Layer IV/III of the enthorinal cortex. Slice viability was tested by recording responses to single or paired stimuli (0.1ms, 1-10V, 50ms interval) in the Schaffer collaterals, delivered using a stimulus isolator in constant voltage mode (ISO Flex, AMPI Instruments, Jerusalem, Israel) controlled by a Master-8 (AMPI Instruments, Israel).

Bipolar stimulation electrodes (self-manufactured) were made of platinum wires, 50µm diameter, and 200µm tip separation. Recordings were digitised at 10 Hz (and low pass filtered at 3 kHz) using CED 1401 interface and Spike 2 software (Cambridge Electronic Design, UK). Slices were perfused with aCSF with no added magnesium at 36 ± 0.5°C (flow rate: ~1.8 ml/min). And after 1 to 2 hours the magnesium removal induced recurrent short discharges (RSDs) in the hippocampus (CA3) and seizure-like events (SLEs) in the medial entorhinal cortex (mEC). After some time (≈ 0,5 hour) the SLE activity changed to late recurrent discharges (LRDs) in the mEC, with a frequency of about 14 ± 3 per min, and duration of 1–3 s. Taurine (5mM) application completely blocked the LRDs, as described in (Kirchner, et al. 2003).

Establishing the procedures: In order to establish this protocol in our laboratory some alterations had to be made, either because of the lack of equipment or due to different apparatus.

Wistar rats were decapitated under deep halothane anesthesia. An agar block had to be mounted in the slicing stage (VT1000 S; Leica, Germany) of the vibratome in order to stabilize the tissue for cutting.

Although a Haas type interface chamber (Harvard Apparatus, Inc) existed in the laboratory, it had never been mounted or used. So it took 2 to 3 weeks to set up the chamber. Furthermore, due to its design, and the layout of the dissecting and electrophysiology separated rooms, it was not possible to immediately transfer the slices from the vibratome to the chamber. As so after slicing, each slice was removed from the vibratome using a Pasteur pipette with the bulb placed over the narrow opening and stored in a home made storage (Wang and Kass 1997). Slices were incubated in aerated ACSF at 35^{o}C for 30 minutes and then allowed to recover at room temperature (RT) (22-24^{o}C) for at least 60 to 90 minutes before starting the experiments. Thus, ensuring the recovery of swollen neurons and mitochondria, caused by ischemia and physical trauma during preparation.

After recovery, slices were transferred to the interface chamber and let to adapt for 20 minutes.

Extracellular field potential recordings were amplified by Axoclamp 2B (Axon Instruments, NY, USA), digitized with a BNC-2110 (National Instruments, USA) and stored on a personal computer for subsequent analysis. Data acquisition was carried out by WinLTP 0.96 (Anderson, 1991-2009) software program in continuous acquisition mode (Anderson and Collingridge 2007). The DIgidata

BNC-2110, that allowed acquisition in continuous mode, was ordered and arrived around July. No simultaneous recording was possible since the setup only has one recording headstage, thus recordings were made preferably from the mEC.

Viability of the slices was only determined by external appearance of the slice or by the onset of epileptiform activity, in the case of free Mg^{2+} aCSF-perfused slices, since no adequate stimulation electrode (bipolar platinum wires) was available in the laboratory.

In order to make the induction of epileptiform activity easier and more reliable in our setup we had to slightly increased K^{+} concentration, thereby increasing excitability, (5 mM vs 3 mM in standard aCSF) in the Free Mg^{2+} aCSF.

Despite the changes in the protocol and time-consuming installment process, I hereby report that it is now possible in our laboratory to induce epileptiform activity via removal of magnesium from the aCSF. We are now able to record recurrent short discharges (RSDs) in the hippocampus (CA1) and both seizure-like events (SLEs) and late recurrent discharges (LRDs) in the mEC.

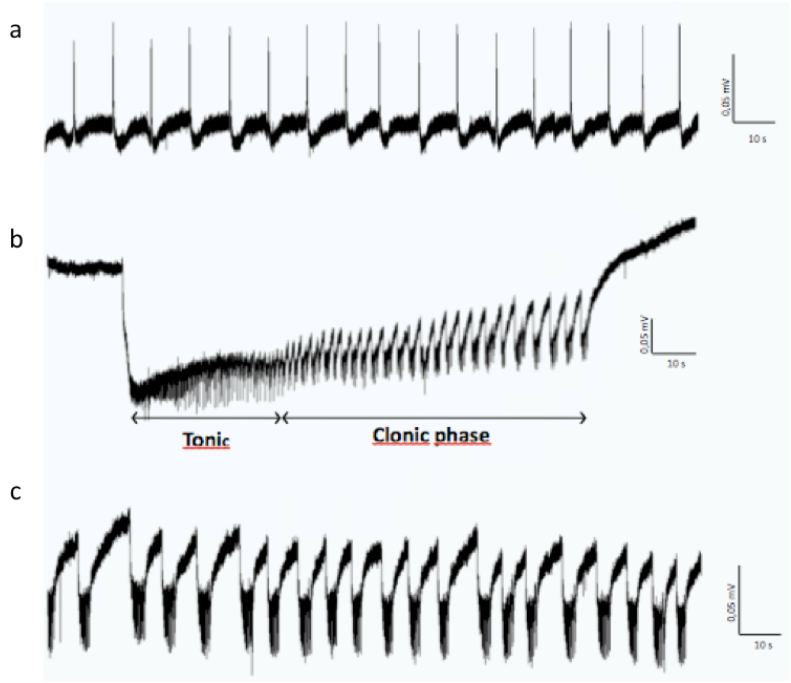

Figure 1: Free Mg^{2+}-induced epileptiform activity recorded in our laboratory. **a)** Recurrent short discharges (RSDs) in CA1. **b)** Seizure-like events (SLEs) in the medial enthorinal cortex (mEC). **c)** Late recurrent discharges (LRDs) in the mEC.

Once again I would like to thank the NENS Office for this opportunity to extend my experimental skills and experience healthy exchange of scientific knowledge that allowed this technique to be successfully implemented in our laboratory. And allowed me to complete my master thesis with very interesting results. It is not enough to acknowledge the accessibility, knowledge and kindness of Dr. Heinemann and his group in having me in the laboratory.

Table 1: Expenses during Trainig stay at Charité University in Berlin

Item	Date	Price (€)
Transportation		
Public transport ticket from the Airport	14.04.11	2,3
Public transport ticket	15.04.11	2,3
Monthly public transport ticket	15.04.11 - 14.05.11	74
Public transport ticket	16.05.11	2,3
4 Trip tickect	16.05.11 - 18.05.11	8,2
Iberia Flight	14.04.11; 18.05.11	297,59
		386,69
Housing		
Room rent		
Rent	14.04.11 - 18.05.11	450
		836,69
Food and Essentials		
Lunchs		
La Luz (Oudenarder Straße 16, 13347, Berlin)	20.04.11	6,50
	21.04.11	1,04
	21.04.11	1,93
	27.04.11	1,78
	29.04.11	2,28
	02.05.11	3,46
	03.05.11	1,69
	04.05.11	2,79
Netto Marken-Discount (Oudenarder Straße 14, 13347, Berlin)	06.05.11	2,95
	09.05.11	1,65 + 0,91
	10.05.11	3,23
	11.05.11	1,11
	11.05.11	2,00
	12.05.11	3,77 + 0,32
	13.05.11	2,33
	17.05.11	1,08
	19.04.11	1,74 + 2,49
		45,05
		881,74
Groceries		
Rewe (Ostbahnhof 9, 10243 Berlin)	22.04.11	4,96
Real (Treptow Park 14, Berlin)	27.04.11	3,40
Kauf Markt (Karl Marx Allee 116, 10243 Berlin)	27.04.11	6,69
Aldi (Rudersdorfer Straße 65, Berlin)	29.04.11	28,47
Kaiser's (Revaler Straße 2, 10243 Berlin)	30.04.11	4,48
Kauf Markt (Karl Marx Allee 116, 10243 Berlin)	03.05.11	12,48
Rewe (Ostbahnhof 9, 10243 Berlin)	08.05.11	4,26
Aldi (Oudenarder Straße 15, 13347, Berlin)	11.05.11	3,38
Rewe (Ostbahnhof 9, 10243 Berlin)	13.05.11	6,88
Netto (Prenzlauer Berg, 10405 Berlin)	13.05.11	9,04
		84,04
Total		965,78
		965,78

*Note: Lunches were mainly purchased in the supermarket (Netto Marken-Discount) near the laboratory (Oudenarder Straße 14, 13347, Berlin). Here is just an approximate estimation of expenses, especially regarding lunches and groceries since I didn't keep every supermarket's receipt.
Note: All proof/receipts are sent in attachment via e-mail.

References:

Anderson, William W, e Graham L Collingridge. "Capabilities of the WinLTP data acquisition program extending beyond basic LTP experimental functions." *Journal of Neuroscience Methods*, n.º 162 (2007): 346-356.

Kirchner, A., J. Breustedt, B. Rosche, U. F. Heinemann, e V. Schmieden. "Effects of taurine and glycine on epileptiform activity induced by removal of Mg2+ in combined rat entorhinal cortex-hippocampal slices." *Epilepsia* 44 (2003): 1145-52.

Wang, Ting, e Ira S Kass. *Preparation of Brain Slices.* Vol. 72, em *Neurotransmitter Methods*, de Richard C Rayne, 1-14. Totowa, NJ : Humana Press Inc , 1997.